Na₄EDTA-Assisted Sub-/Supercritical Fluid Extraction Procedure for Quantitative Recovery of Polar Analytes in Soil

Fengmao Guo, Qing X. Li,* and Jocelyn P. Alcantara-Licudine

Department of Environmental Biochemistry, University of Hawaii at Manoa, Honolulu, Hawaii 96822

Supercritical carbon dioxide (SC-CO₂) is effective in extracting nonpolar and slightly polar chemicals from soils. However, pure SC-CO₂ is unsatisfactory for recovering polar chemicals in soils. A simple supercritical fluid extraction (SFE) procedure was developed to quantitatively recover polar and nonpolar chemicals from soils. The polar chemicals tested were aromatic acids and phenols. The nonpolar and slightly polar chemicals used as model compounds were common pesticides and environmental pollutants such as polycyclic aromatic hydrocarbons. The procedure required pretreatment of the samples with 15% water (g/g), 5% (ethylenedinitrilo)-tetraacetic acid tetrasodium salt (Na₄EDTA) (g/g), and 50% methanol (mL/g) prior to extractions using SC-CO₂ at 60 °C and 34.5 MPa. Recoveries ranged from 90 to 106% for the aromatic acids using the Na₄EDTA-assisted SFE compared with only 7−63% recoveries of the corresponding chemicals when no Na₄EDTA was used. The method quantitatively extracted 2,4-D and its close analogues aged in the soil for 2−30 days. The Na₄EDTA-assisted SFE was also adequate for extracting phenolic analytes including picric acid and pentachlorophenol with recoveries from 85 to 104%. Na₄EDTA is a good enhancer for extraction of the 29 analytes representing a wide range of polarity from the soil using SC-CO₂. The method is valuable for the analysis of parent pollutants and transformed products, particularly oxygen-borne metabolites in the environment.

Complete extraction of polar organic residues in complex environmental media such as soils is difficult.[1,2] Conventional solvent extractions such as Soxhlet often yield low recoveries and complex extracts that require extensive sample cleanup for quantitative analysis.[2] Supercritical fluid extraction (SFE) has recently received increasing interest for extracting a variety of compounds in soils. SFE minimizes organic solvent consumption and often can achieve equivalent or better recoveries than the solvent extractions. SFE also gives clean extracts, which often can avoid a cleanup step for analyte detection.

Supercritical carbon dioxide (SC-CO₂) is widely used in SFE and is capable of extracting nonpolar or slightly polar compounds from complex media with considerable selectivity,[3−7] but is less effective in recovering large and polar molecules because of the properties of SC-CO₂.[8−13] Many polar solvents have been added in SC-CO₂ to alter its polarity and subsequently to improve extraction efficiency of some chemicals such as herbicides from soils[8,14−16] and house dust.[17] However, adding solvents directly in CO₂ requires instrumental modification. The copresence of solvents in CO₂ increases the critical temperature (Tc) and pressure (Tp). Therefore, only a small percentage of cosolvents in SC-CO₂ is practically allowed. Addition of water to soils (up to 20%, w/w) prior to SFE with SC-CO₂ improved the recoveries of polar analytes.[18−20] Water alone is not a satisfactory modifier for the strongly soil-affinitive compounds. Cosolvents such as methanol (MeOH) have significantly improved the SFE efficiency.[9,12,15,18] However, cosolvents in SC-CO₂ are often inadequate for quantitative extraction of polar compounds, particularly when aged in soils.[12,15,21] Few techniques including SFE gave acceptable recoveries for extracting certain compounds in soils.[22]

Derivatization of polar analytes or sorption sites in situ or off-line has recently received considerable attention.[11,14,23] The derivatization approach can be effective but requires considerable

(1) Chester, T. L.; Pinkston, J. D.; Raynie, D. E. *Anal. Chem.* **1998**, *70*, 301R−319R.
(2) Dean, J. R. *J. Chromatogr., A* **1996**, *754*, 221−233.
(3) Alexandrou, N.; Pawliszyn, J. *Anal. Chem.* **1989**, *61*, 2770−2776.
(4) Onuska, F. I.; Terry, K. A. *J. High Resolut. Chromatogr.* **1991**, *41*, 829−834.
(5) Wong, J. M.; Li, Q. X.; Hammock, B. D.; Seiber, J. N. *J. Agric. Food Chem.* **1991**, *39*, 1802−1807.
(6) Hawthorne, S. B.; Miller, D. J. *Anal. Chem.* **1994**, *66*, 4005−4012.
(7) Reindl, S.; Höfler, F. *Anal. Chem.* **1994**, *66*, 1808−1816.
(8) McNally, M. E. P.; Wheeler, J. R. *J. Chromatogr.* **1988**, *447*, 53−63.
(9) Snyder, J. L.; Grob, R. L.; McNally, M. E.; Oostdyk, T. S. *J. Chromatogr. Sci.* **1993**, *31*, 183−191.
(10) Steinheimer, T. R.; Pfeiffer, R. L.; Scoggin, K. D. *Anal. Chem.* **1994**, *66*, 645−650.
(11) Field, J. A. *J. Chromatogr., A* **1997**, *785*, 239−249.
(12) Yuan, H.; Olesik, S. V. *J. Chromatogr., A* **1997**, *764*, 265−277.
(13) Field, J. A.; Monohan, K.; Reed, R. *Anal. Chem.* **1998**, *70*, 1956−1962.
(14) Rochette, E. A.; Harsh, J. B.; Hill, H. H., Jr. *Talanta* **1993**, *40*, 147−155.
(15) Koskinen, W. C.; Cheng, H. H.; Jarvis, L. J.; Sorenson, B. A. *Int. J. Environ. Anal. Chem.* **1995**, *58*, 379−385.
(16) Rochette, E. A.; Koskinen, W. C. *Soil Sci. Soc. Am. J.* **1996**, *60*, 453−460.
(17) Reighard, T. S.; Olesik, S. V. *Anal. Chem.* **1997**, *69*, 566−574.
(18) Locke, M. A. *J. Agric. Food Chem.* **1993**, *41*, 1081−1084.
(19) Fahmy, T. M.; Paulaitis, M. E.; Johnson, D. M.; McNally, M. E. P. *Anal. Chem.* **1993**, *65*, 1462−1469.
(20) Brady, B. O.; Kao, C. P. C.; Dooley, K. M.; Knopf, F. C.; Gambrell, R. P. *Ind. Eng. Chem. Res.* **1987**, *26*, 261−268.
(21) Khan, S. U. *J. Agric. Food Chem.* **1995**, *43*, 1718−1723.
(22) Lopez-Avila, V.; Young, R.; Teplitsky, N. *J. AOAC Int.* **1996**, *79*, 142−156.
(23) Hawthorne, S. B.; Miller, D. J.; Nivens, D. E.; White, D. C. *Anal. Chem.* **1992**, *64*, 405−412.

Table 1. Basic Properties of the Leilehua Soil

parameter	description
taxonomy	ustic kanhaplohumult
pH (1:1 H$_2$O)	4.66
sand, %	1.7
silt, %	12.0
clay content, %	86.3
organic carbon content, %	4.4
dominant mineralogy	gibbsite, goethite, hematite, amorphous material, kaolinite, and illite

time and effort. Samples with high concentrations of reactive matrix components require multiple derivatization/extraction steps to achieve quantitative recoveries.[23] Side products often make quantification difficult.[17]

Some polar compounds strongly interact with the soil components. Such interactions make the polar chemicals in soils unextractable by pure SC-CO$_2$.[9,14] However, if the interaction can be chemically overcome prior to extraction, subsequent SFE may result in good recoveries of polar analytes. Chemical modifiers have been studied to enhance the extraction efficiency for a few polar compounds. These modifiers included derivatizing reagents, solvents, inorganic salts, bases, and acids. A recent study reported that (ethylenedinitrilo)tetraacetic acid tetrasodium salt (Na$_4$EDTA) significantly improved recoveries of xanthene dyes in soils using SC-CO$_2$.[25] The chelating agent Na$_4$EDTA may perform ligand formation, ionic displacement, and site covering. Based on these hypotheses, Na$_4$EDTA may enhance the extractability of other polar compounds from soils. This study thoroughly examined the Na$_4$EDTA-assisted SFE method for complete recovery of a wide range of aromatic acids, phenols, and neutral compounds fortified in a clayey soil.

EXPERIMENTAL SECTION

Soil. The soil used in this study was the Leilehua series collected from the Waiawa Correctional Facility, Oahu, HI. The soil was sampled from 0 to 15 cm from the soil surface. A composite sample (2 kg) was air-dried and ground to pass a 0.84-mm sieve (U.S. 20 mesh). Water content was determined by drying a ground sample (10 g) overnight at 105−110 °C. The ground samples were stored in capped glass jars. The basic properties of the soil are described in Table 1. The soil is a highly weathered, acidic, and clayey Ultisol. Because of the mineral composition and high clay content, it is considered a variable-charge soil that is highly affinitive to a variety of anionic compounds.

Reagents. The compounds selected for this study are shown in Table 2. 2,4-D, 2,4-DB, 2,4,5-T, dicamba, and picloram were obtained from the U.S. Environmental Protection Agency (Beltsville, MD). Benzoic acid, picric acid, 2-naphthoic acid, 1-naphthylacetic acid, 1-naphthol, phenol, pentachlorophenol, 9-phenanthrol, resorcinol, 1-hydroxypyrene, anthrancene, benzo[*a*]pyrene, naphthalene, phenanthrene, pyrene, pentachlorobenzene (PCB) and pentachloronitrobenzene (PCNB) were purchased from Aldrich

Chemical Co. (Milwaukee, WI). Alachlor, bromacil, chlorpyrifos, dacthal (DCPA), hexazinone, metolachlor, and metribuzin were purchased from Chem Services, Inc. (West Chester, PA). Na$_4$EDTA was purchased from Matheson Coleman and Bell (Norwood, OH). Anhydrous sodium sulfate purchased from Mallinckrodt, Inc. (Chesterfield, MO) was baked overnight at 400 °C. Water for capillary zone electrophoresis (CZE) was laboratory distilled and filtered through a Milli-Q water purification system (Millipore Co., Bedford, MA) set at 18 MΩ·cm. Sodium hydroxide, boric acid, HPLC-grade water, and Optima-grade MeOH were purchased from Fisher Scientific (Pittsburgh, PA). Sodium borate decahydrate (Na$_2$B$_4$O$_7$·10H$_2$O) was obtained from J. T. Baker Inc. (Phillipsburg, NJ). Compressed CO$_2$ (99.5% purity) for SFE and medical air for CZE were from Air Liquide (Honolulu, HI). High-purity helium was obtained from Gaspro (Honolulu, HI).

Standard stock solutions (1 mg/mL) of all polar compounds (acids and phenols) were prepared in MeOH. Standard stock solutions of all nonpolar and slightly polar compounds were prepared in methylene chloride at 1 mg/mL. All standard solutions were kept at 4 °C.

SFE Instruments. An Isco SFX 2-10 extractor (Lincoln, NE) was connected to an Isco 260D syringe pump with a cooling jacket cooled by a Brinkmann RM6 circulator set at 10 °C. The SC-CO$_2$ flow was controlled at 1.5−2 mL/min using a stainless steel capillary restrictor (25 cm × 300 μm o.d.) which was housed in a restrictor heater at 70 °C. Both flow rate and total SC-CO$_2$ volume were recorded as direct reading from the instrument.

SFE Optimization. Soil (2 g equiv air-dried) was spiked with 0.1 mL of 100 μg/mL 2,4-D stock solution. Water in soil was adjusted to a final content of 6.2 (air-dry)−20%. The sample was held at ambient temperature for 1 h and mixed two to three times during this equilibration period. The spiked sample was thoroughly mixed with 0−7% (0−70 mg/g of soil) Na$_4$EDTA and quantitatively transferred to a 2.5-mL extraction cell. MeOH was then added to each cell (0.25−0.625 mL/g of soil). The pressure was 48.3 MPa (7000 psi). The temperatures of the extraction chamber and collection capillary heater were 60 and 70 °C, respectively. A ratio of water, Na$_4$EDTA, and MeOH was optimized by varying one parameter at a time with the others held at experimentally optimum values. The ratio is a close approximation to the true optimum value.

SFE of Freshly Spiked Samples. A sample (2 g equiv air-dried) was weighed into a 10-mL beaker and spiked with appropriate amounts of the standard solutions. Water content was adjusted to 15%. The sample was mixed well and covered with aluminum foil. After the sample was incubated for 1 h, Na$_4$EDTA (100 mg) was added, well mixed, and covered with Al foil. After 1-h equilibration at ambient temperature, the sample was transferred to a 2.5-mL extraction cell. One milliliter of MeOH (50%, mL/g) was then added. The samples underwent a 5-min static extraction, and a dynamic extraction step to collect 30 mL of SC-CO$_2$. Additional MeOH (1 mL) was added in the sample after the extraction cell was cooled at ambient temperature. The sample was re-extracted by the same static and dynamic extractions as described above. During these extractions, the pressure was 34.5 MPa (5000 psi). The temperatures of the extraction chamber and collection capillary heater were 60 and 70 °C, respectively. The acids and phenols were collected in 15 mL of MeOH. The

(24) Lopez-Avila, V.; Dodhiwala, N. S.; Beckert, W. F. *J. Agric. Food Chem.* **1993**, *41*, 2038−2044.

(25) Alcantara-Licudine, J. P.; Kawate, M. K.; Li, Q. X. *J. Agric. Food Chem.* **1997**, *45*, 766−773.

Table 2. Chemicals Examined in This Study

common name	λ_{max}, nm	chemical name
	Polar Analytes (Analyzed by CZE)	
2,4-D	230	2,4-dichlorophenoxyacetic acid
2,4-DB	230	4-(2,4-dichlorophenoxy)butyric acid
2,4,5-T	230	2,4,5-trichlorophenoxyacetic acid
dicamba	230	3,6-dichloro-2-methoxybenzoic acid
picloram	346	4-amino-3,5,6-trichloropicolinic acid
benzoic acid	227	benzoic acid
2-naphthoic acid	234	2-naphthoic acid
1-naphthylacetic acid	280	1-naphthylacetic acid
phenol	272	phenol
PCP	275	pentachlorophenol
resorcinol	255	1,3-benzenediol
1-naphthol	296	1-naphthol
9-phenanthrol	248	9-phenanthrol
1-hydroxypyrene	241	1-hydroxypyrene
picric acid	254	2,4,6-trinitrophenol
	Slightly Polar Analytes (Analyzed by GC−NPD or ECD)	
alachlor		2-chloro-2′,6′-diethyl-N-(methoxymethyl)acetanilide
bromacil		5-bromo-3-sec-butyl-6-methyluracil
hexazinone		3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4-(1H,3H)-dione
metolachlor		2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide
metribuzin		4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5-(4H)-one
dacthal (DCPA)		dimethyl tetrachloroterephthalate
PCB		pentachlorobenzene
PCNB		pentachloronitrobenzene
chlorpyrifos		O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl)phosphorothioate
	Nonpolar Analytes (Analyzed by GC-FID)	
naphthalene		naphthalene
phenanthrene		phenanthrene
anthrancene		anthrancene
pyrene		pyrene
benzo[a]pyrene		benzo[a]pyrene

remainders of the analytes were collected in CH_2Cl_2. During the extraction of polycyclic aromatic hydrocarbons (PAHs), glass fiber was used as a barrier to fill the upper part of the collection tubes. This barrier prevented spills of trapping CH_2Cl_2 and loss of relatively volatile PAHs.

SFE of Aged Samples. Soil (2 g equiv air-dried) in 10-mL beakers was spiked with 2,4-D, 2,4-DB, 2,4,5-T, or dicamba at 5 μg/g in 15 replicates. After moisture was adjusted to 15%, the samples were mixed well, covered with Al foil, sealed with Parafilm, and stored at −20 °C. Five spiked samples of each compound were extracted at 2, 7, or 30 days after fortification. Prior to extraction, each sample was treated with 100 mg of Na_4EDTA, mixed well, and covered with Al foil. After a 1-h equilibration, the samples were extracted in the same manner as described above.

Capillary Zone Electrophoretic Determination of Acids and Phenols. The extracts were filtered through a 100-mL glass syringe with disposable GHP Acrodisc filters (25 mm × 0.45 μm) (Gelman Sci., Ann Arbor, MI) to 125-mL round flasks. MeOH in the extracts was reduced to 1−2 mL using a rotary evaporator. The concentrated extracts were quantitatively transferred to 5-mL graduated centrifuge tubes with MeOH. The extracts were stored at 4 °C for later analysis.

The acids and phenols were determined by capillary zone electrophoresis (CZE) with an ultraviolet/visible detector (Table 2). The CZE system was a Dionex CES I equipped with a Dionex 4400 integrator (Dionex Co., Sunnyvale, CA). The capillary was fused silica (75 μm i.d. × 67 cm). The running buffer for the acids was 10 mM $Na_2B_4O_7$/50 mM H_3BO_3 (pH 8.5). A buffer of 10 mM $Na_2B_4O_7\cdot10H_2O$/22.7 mM NaOH (pH 10.5) was used for the determination of all phenols. Running buffers were filtered through a 47-mm, 0.45-μm Whatman Nylon filter (Whatman, Maldstone, UK) prior to use. Polarity was set at the "+" position (positive at detector end). Gravity injection was held at a height of 50 mm for 10 s. Potential was constant at 20 000 V, and current limit was 300 μA. Run time was from 4 to 6 min depending on the migration time of the analytes. The destination vial and capillary were flushed with the running buffer for 6 and 120 s, respectively. The capillary was rinsed once with the running buffer between runs. Absorption maximums of the analytes (Table 2) were determined by scanning a 20 ppm solution of each compound in MeOH using a Shimadzu UV160U spectrophotometer.

Gas Chromatographic Determination of Nonpolar and Slightly Polar Compounds. The extracts of anthrancene, benzo-[a]pyrene, naphthalene, phenanthrene, and pyrene were dried over anhydrous Na_2SO_4 (10 g). The extracts were filtered through GHP Acrodisc filters (25 mm × 0.45 μm) to 125-mL round flasks. The extracts were concentrated to 1−2 mL using a rotary evaporator and completely transferred to 5-mL graduated centrifuge tubes. The final volume was adjusted to 2 mL using a mild stream of nitrogen gas. The extracts were stored at 4 °C until GC analysis.

The extracts of alachlor, bromacil, chlorpyrifos, dacthal, hexazinone, metolachlor, metribuzin, PCB, and PCNB were dried over anhydrous Na_2SO_4, filtered, and concentrated to 1−2 mL as described above. A 25-mL aliquot of methyl $tert$-butyl ether (MTBE) was added, and the resultant mixture was concentrated to 1−2 mL. This process was repeated once. The concentrated
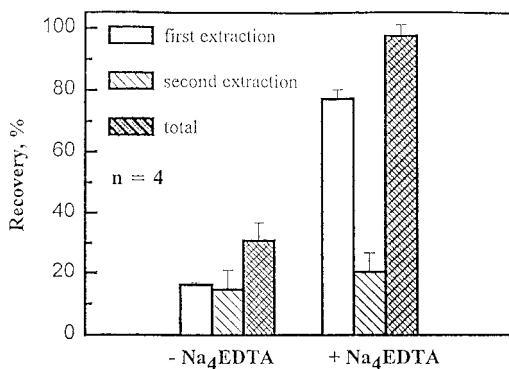
Figure 1. Effect of Na₄EDTA on extraction efficiency of 2,4-D spiked in soil as examined by the recovery in fractions of two consecutive extractions. Soil (2 g) was treated with 15% water (g/g), 0 or 5% Na₄EDTA (g/g), and 1 mL of MeOH (50%, mL/g) prior to the first extraction. One milliliter of MeOH alone was added prior to the second extraction. Each extraction had a 5-min temperature equilibration, a 5-min static extraction, and a dynamic extraction step to collect 30 mL of SC-CO₂. Pressure was 48.3 MPa, and temperature was 60 °C.

Figure 2. Effect of soil moisture on 2,4-D recovery. Soil (2 g) was treated with 5% Na₄EDTA (g/g), 1 mL of MeOH (50%, mL/g), and an appropriate amount of water (6–20%, g/g) prior to extraction. After a 5-min temperature equilibration in the extraction chamber, the fortified samples were statically extracted for 5 min and dynamically extracted with 30 mL of SC-CO₂ at 48.3 MPa and 60 °C. One milliliter of MeOH alone was added prior to the subsequent static and dynamic extractions at the above condition. The extracts were combined.

extracts were completely transferred to 5-mL graduated centrifuge tubes and adjusted to 5 mL with MTBE. The extracts were stored at 4 °C until GC analysis.

An HP 5890 GC equipped with a nitrogen−phosphorus detector (NPD) was used to determine alachlor, bromacil, hexazinone, metolachlor, and metribuzin. An electron capture detector (ECD) was used to determine chlorpyrifos, dacthal, PCB, and PCNB. In both cases, the column was a DB-5 (0.25 μm × 0.25 mm i.d. × 30 m, J&W Scientific). The injection volume was 2 μL in splitless mode with a 45-s delay. The injector and detector temperatures were 250 and 300 °C, respectively. Helium carrier gas flow was 30 cm/s measured at 50 °C. For the former group of chemicals, oven temperature was programmed from 100 to 225 °C at 5 °C/ min and then to 300 °C at 20 °C/min and held at 300 °C for 5 min. For the latter group of chemicals, oven temperature was programmed from 100 to 300 °C at 20 °C/min and held at 300 °C for 5 min.

PAHs were analyzed with an HP 5890 Series II equipped with a flame ionization detector (FID). The column was a DB-5.625 (0.25 μm × 0.25 mm i.d. × 30 m). The helium carrier gas flow was 36 cm/s measured at 50 °C. Oven temperature was programmed from 50 to 250 at 10 °C/min and then to 300 °C at 20 °C/min and held at 300 °C for 7.5 min. The injection volume was 2 μL at splitless mode with 45-s delay. The injection and detector temperature was 275 °C.

RESULTS AND DISCUSSION

SFE Optimization. The chlorophenoxyacetic acid herbicide 2,4-D was used as a model chemical to optimize SFE conditions. In preliminary experiments, the fortified soil samples were modified with 15% water (g/g), 5% Na₄EDTA (g/g), and 50% MeOH (mL/g). These modifier ratios were chosen on the basis of our previous work.[25] The sample was extracted using SC-CO₂ following a two-step procedure, and the extracts were collected in two fractions. 2,4-D in the first fraction accounted for ∼80% of the spiked amount from the Na₄EDTA-modified samples compared with only 16% recovery when no Na₄EDTA was added (Figure 1).
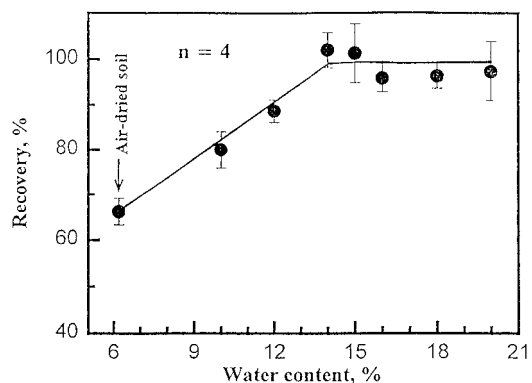
Na₄EDTA significantly enhanced the 2,4-D recovery, and two consecutive extractions were sufficient for quantitatively recovering 2,4-D. MeOH also played an important role in improving the recoveries. Additional MeOH is necessary to recover the remaining analyte in the second extraction. Increasing SC-CO₂ volume and extraction time did not further increase the recovery after 20 mL of SC-CO₂ (48.3 MPa and 60 °C) passed through the sample. This suggested that incomplete recoveries were not due to SC-CO₂ diffusion limitations or kinetic effects and was consistent with the findings in previous studies.[15,18,19] The effects and appropriate ratios of Na₄EDTA, water, and MeOH were further examined for quantitative recoveries of 2,4-D by varying one parameter at a time while holding the others constant.

Soil moisture varied from 6.2 to 20% while Na₄EDTA and MeOH were 5 (g/g) and 50% (mL/g), respectively. Water greatly affected 2,4-D recovery from the soil (Figure 2). Extraction of the air-dried soil (6.2% water) with 5% Na₄EDTA and 50% MeOH gave a recovery of 67%. When water content was 14−15%, the method completely recovered the added 2,4-D. When water content was greater than 15%, 2,4-D recoveries were 96−97%. However, filter blockage, and frequent outlet plugging due to ice formation within the capillary restrictor occurred. Therefore, soil water content greater than 15% was not desirable.

Na₄EDTA is critical to 2,4-D extraction from the soil by SFE (Figure 3). Recoveries of 2,4-D increased from 36% without the aid of Na₄EDTA to a quantitative recovery when Na₄EDTA was added at 5% or greater. When Na₄EDTA was 4% (40 mg/g soil), the recovery was 89%. When Na₄EDTA was less than 4%, low recoveries (36−72%) and large variations were observed. The efficiency of Na₄EDTA is related to its quantity and homogeneous distribution in soil. When Na₄EDTA was greater than 5%, Na₄EDTA and/or its complexes deposited on the filters. Frequent plugging occurred and sonication was necessary to remove the precipitates.

MeOH is an effective modifier for 2,4-D recovery. The MeOH/ soil ratios were examined in a range of 0.25−0.625 mL/g of soil (25−62.5%, mL/g) (Figure 4). Adequate MeOH/soil ratios ranged from 0.5 to 0.6 mL/g (50−60%). MeOH/soil ratios greater than
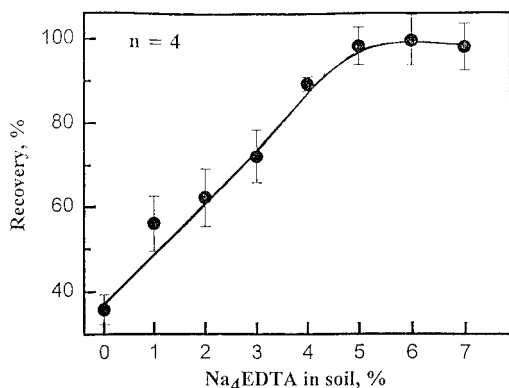
Figure 3. Optimization of Na₄EDTA ratio for 2,4-D extraction. Soil moisture was 15%, and the extraction conditions were the same as described in Figure 2.
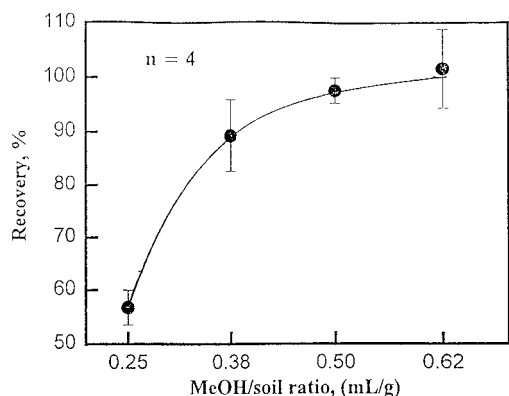

Figure 4. Effect of MeOH on 2,4-D recovery. Soil was treated with 15% water, 5% Na₄EDTA (g/g), and different amounts of MeOH prior to extraction. The SFE conditions were the same as described in Figure 2.
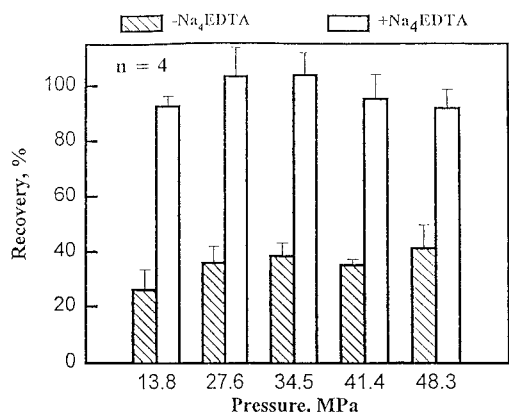

Figure 5. Effect of pressure on 2,4-D recovery. The SFE conditions, except for pressure, were the same as described in Figure 2.

0.63 gave cloudy extracts due to coextractives that blocked the filter and capillary restrictor.

When the sample was treated with 15% water and 50% MeOH (mL/g), the effect of pressure on 2,4-D recovery was investigated with and without the addition of 5% Na₄EDTA. Pressure between 13.8 and 48.3 MPa was not critical to 2,4-D extraction (Figure 5). The extraction probably proceeded in subcritical (pressurized liquid) and supercritical phases during the static step and in supercritical solution during the dynamic extraction. 2,4-D recoveries were 92–104% from the Na₄EDTA-treated samples when

Table 3. Recoveries of Polar and Nonpolar Compounds from a Clayey Soil

	recovery ± SD,[a] %	
analytes	without Na₄EDTA	with Na₄EDTA
Chlorophenoxy and Aromatic Acid (Spike Level, 5 μg/g of Soil)		
2,4-D	35.8 ± 3.5	97.5 ± 2.4
2,4-DB	63.2 ± 6.2	100.3 ± 5.3
2,4,5-T	49.8 ± 5.5	102.4 ± 1.3
dicamba	62.3 ± 6.8	106.3 ± 7.8
picloram	7.1 ± 1.2	95.0 ± 6.4
benzoic acid	44.1 ± 2.6	100.3 ± 3.9
2-naphthoic acid	56.3 ± 4.6	99.7 ± 3.4
1-naphthylacetic acid	45.2 ± 3.1	90.3 ± 4.6
Phenols (Spike Level, 5 μg/g)		
phenol[b]	96.8 ± 3.8	96.3 ± 5.2
resorcinol[b]	97.3 ± 4.3	102.0 ± 0.9
pentachlorophenol	96.5 ± 6.4	103.8 ± 1.6
1-naphthol	92.3 ± 6.1	94.4 ± 4.0
9-phenanthrol	62.9 ± 4.5	96.2 ± 1.8
1-hydroxypyrene	67.3 ± 7.1	98.7 ± 4.9
picric acid	22.3 ± 4.2	85.3 ± 14.9
Chloroacetanilide and Nitrogen Heterocyclic Herbicides (Spike Level, 0.1 μg/g)		
alachlor	98.2 ± 6.5	98.5 ± 3.8
bromacil	92.6 ± 10.9	114.0 ± 7.4
hexazinone	99.9 ± 14.5	100.5 ± 10.9
metolachlor	100.8 ± 7.4	102.1 ± 5.0
metribuzin	98.4 ± 11.4	99.9 ± 10.2
Chlorinated and Organophgosphorus Compounds (Spike Level, 0.01 μg/g)		
chlorpyrifos	93.1 ± 3.9	103.7 ± 5.3
dacthal	105.4 ± 5.9	102.7 ± 3.8
pentachlorobenzene	92.3 ± 4.3	100.1 ± 5.6
pentachloronitrobenzene	93.5 ± 5.6	92.3 ± 5.1
Polycyclic Aromatic Hydrocarbons (Spike Level, 10 μg/g)		
naphthalene	95.1 ± 5.1	93.4 ± 8.4
phenanthrene	99.5 ± 8.0	99.1 ± 12.7
anthrancene	94.1 ± 8.5	97.3 ± 9.4
pyrene	93.2 ± 11.8	101.3 ± 9.8
benzo[a]pyrene	85.1 ± 14.8	94.9 ± 3.2

[a] SD, standard deviation; $n = 4$. [b] Phenol and resorcinol were spiked at 10 μg/g of soil.

pressure varied from 13.8 to 48.3 MPa (2000−7000 psi). When no Na₄EDTA was added, 2,4-D recoveries increased from 22 to 40% as the pressure increased from 13.8 to 48.3 MPa. However, extractions at high pressures (41.4 and 48.3 MPa) often caused collection spills and sample compactness. Optimum pressures for 2,4-D extraction ranged from 27.6 to 34.5 MPa (4000−5000 psi). Therefore, a pressure of 34.5 MPa (5000 psi) was used for the remainder of the study.

Polar Compounds (Acids and Phenols). The optimized procedure for 2,4-D was used to extract eight carboxylic acids and seven phenols (Table 3). These carboxyl and hydroxyl compounds strongly interact with the soil mineral and organic colloidal surfaces. Acids are strongly retained into the soil matrix with variable-charge minerals (e.g., oxides and allophane) and organic components.[26] For example, 2,4-D is adsorbed by iron oxides probably by coordination of the carboxylic group to a surface Fe^{3+} ion to form complexes.[26] Carboxylic acids and phenols can also bond to variable-charge minerals via direct

---

(26) McBride, M. B. *Environmental Chemistry of Soils*; Oxford: New York, 1994.

Table 4. Recoveries of Four Acidic Pesticides Aged in a Clayey Soil

		recovery ± SD,[c] %			
aging,[a] days	modifier[b]	2,4-D	2,4-DB	2,4,5-T	dicamba
0	MeOH/H$_2$O	35.8 ± 3.5	63.2 ± 6.2	49.8 ± 5.5	62.3 ± 6.8
0	MeOH/H$_2$O/Na$_4$EDTA	97.5 ± 2.4	100.3 ± 5.3	102.4 ± 1.3	106.3 ± 7.8
2	MeOH/H$_2$O/Na$_4$EDTA	96.5 ± 4.4	99.7 ± 3.9	98.1 ± 5.2	96.8 ± 7.5
7	MeOH/H$_2$O/Na$_4$EDTA	95.5 ± 2.7	100.9 ± 13.6	96.6 ± 10.9	97.0 ± 13.3
30	MeOH/H$_2$O/Na$_4$EDTA	93.3 ± 6.7	99.1 ± 1.8	93.6 ± 6.6	96.4 ± 11.0

[a] Samples were spiked at 5 μg/g, mixed well, and immediately analyzed or sealed and incubated at -20 °C in the dark for 2–30 days prior to extraction. [b] SFE conditions were the same as described in Figure 2. The samples were treated with 0 or 5% Na$_4$EDTA (g/g) prior to extraction. [c] SD, standard deviation; $n = 4$ except for freshly spiked 2,4-D samples where $n = 36$.

coordination; therefore, these compounds may be retained to some degree by allophane and oxide minerals of soils. Studies indicated that SC-CO$_2$ alone was not effective in recovering polar analytes from soils. [14,15,17]

The recoveries of the eight spiked acids ranged from 7.1 to 62.3% when the samples were pretreated with the water/MeOH and extracted with SC-CO$_2$. Excellent recoveries (90–106%) were achieved when the samples were pretreated with the Na$_4$EDTA/ water/MeOH mixture and extracted with SC-CO$_2$ (Table 3). Water helps matrix expansion[19] and, consequently, penetration of SC-CO$_2$ and MeOH to remove retained compounds.[18] In the water/ MeOH/SC-CO$_2$ system, the solvating power of SC-CO$_2$ was enhanced by water and MeOH. This mixture was able to effectively remove the weakly bound phenols from the soil matrix but not the strongly bound acids and phenols. Picric acid, 9-phenanthrol, and 1-hydroxypyrene are relatively large molecules and probably are strongly bound with soils. Therefore, the recoveries were low (22–67%) when the extraction was not assisted with Na$_4$EDTA. When Na$_4$EDTA was added, the recoveries increased to 96, 99, and 85% for 9-phenanthrol, 1-hydroxypyrene, and picric acid, respectively. Na$_4$EDTA may disturb the microenvironments where the compounds are sorbed. The EDTA anion may chelate metallic adsorption sites on the soil surfaces as well as compete with the acids for sorption sites. Results from this study demonstrated that SC-CO$_2$ assisted by a mixture of MeOH/water/Na$_4$EDTA is able to quantitatively extract these strongly bound acids and phenols in soils.

Slightly Polar Compounds. The chloroacetanilide and nitrogen heterocyclic herbicides and chlorinated and organophosphorus pesticides and compounds are considered slightly polar chemicals (Table 3). These chemicals are common pollutants found in the environment and were used in this study to evaluate the method applicability as well as to compare with the polar chemicals. There was no difference in the recoveries when no Na$_4$EDTA was used (92–105%) compared with those assisted with Na$_4$EDTA (92–114%).

Nonpolar Compounds. MeOH and water have been successfully used as modifiers for extracting PAH. [7,27–29] The results showed that water/MeOH-assisted SC-CO$_2$ was effective for extracting PAHs from the soil (Table 3). The PAH recoveries except for benzo[a]pyrene were complete (93–101%) with or without pretreatment of the samples by Na$_4$EDTA. Na$_4$EDTA was not critical to the extraction of PAHs although it slightly improved the recoveries of pyrene and benzo[a]pyrene (93 vs 101% and 85 vs 95%, respectively). This is apparently due to the weak interaction between nonpolar compounds and the soil. The water/MeOH-assisted SC-CO$_2$ was powerful enough to displace these chemicals from the soil.

Polar Compounds Aged in Soil. The Na$_4$EDTA-assisted SFE procedure was further examined to recover polar chemicals aged in soil. The interaction between the soil and polar compounds requires time to reach equilibrium. Retention mechanism may also change with time.[15] For this reason, the SFE conditions for extracting compounds from aged soil samples may well be different.[2,15] Our method was applied to extract some highly affinitive polar compounds that were aged in the same soil from 2 to 30 days. The spiked soil samples were kept at -20 °C in the dark. This allowed the system to reach an adsorptive equilibrium while preventing degradation of the analytes during incubation. Recoveries of the four acids aged in the soil for 2–30 days averaged 93–101% (Table 4). No significant difference in the recoveries was found among the three incubation periods and between freshly spiked and aged samples.

CONCLUSIONS

A Na$_4$EDTA-assisted SFE procedure was developed for the extraction of polar chemicals in a clayey soil. The method was optimized with the herbicide 2,4-D and was successfully applied to the extraction of 29 polar, slightly polar, and nonpolar compounds with recoveries ranging from 86 to 106%. This simple two-step extraction required pretreatment of the soil with a mixture of 5% Na$_4$EDTA (g/g), 15% water (g/g), and 50% MeOH (mL/g) and subsequent extraction with 30 mL of SC-CO$_2$. The extracted soil was treated with an additional aliquot of MeOH (50%, mL/g) and re-extracted with another 30 mL of SC-CO$_2$. The recoveries of the eight aromatic acids using the Na$_4$EDTA-assisted SFE ranged from 90 to 106%. The recoveries were only 7–63% without use of Na$_4$EDTA. Among the seven phenols tested, the recoveries ranged from 22 to 97% when Na$_4$EDTA was not added and from 85 to 104% when Na$_4$EDTA was present. Na$_4$EDTA enhanced the recoveries of large and polar phenols. The Na$_4$EDTA-assisted SFE gave recoveries of 93–106% for 2,4-D, 2,4-DB, 2,4,5-T, and dicamba aged in the soil for 2–30 days. The method was also capable of quantitatively recovering 14 slightly polar to nonpolar compounds fortified in the soil. This procedure does not need addition of cosolvents in SC-CO$_2$, thus, is less equipment-

(27) U.S. Environmental Protection Agency, Method 3561, Washington, DC, 1996.
(28) Monserrate, M.; Olesik, S. V. *J. Chromatogr. Sci.* **1997**, *35*, 82–90.
(29) Ashraf-Khorassani, M.; Combs, M. T.; Taylor, L. T. *J. High Resolut. Chromatogr.* **1995**, *18*, 709–712.

demanding. The principle illustrated in this study may be applicable for remediation of chemical pollution sites. The method is also valuable for risk assessment of parent pollutants and transformed products, particularly oxygen-borne metabolites in the environment.